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I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PS 2833 for a patent by MONASH UNIVERSITY as filed on 07 June 2002.

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Seventeenth day of June 2003

JONNE YABSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

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PROVISIONAL SPECIFICATION

for the invention entitled:

"Therapeutic molecules and methods – 2"

The invention is described in the following statement:

THERAPEUTIC MOLECULES AND METHODS – 2

FIELD OF THE INVENTION

The present invention relates generally to the treatment of diseases or conditions resulting from cellular activation, such as inflammatory or cancerous diseases or conditions. In particular, the invention relates to the use of naphthalene derivatives to inhibit the cytokine or biological activity of macrophage migration inhibitory factor (MIF), and diseases or conditions wherein MIF cytokine or biological activity is implicated.

BACKGROUND OF THE INVENTION

MIF is the first identified T-cell-derived soluble lymphokine. MIF was first described as a soluble factor with the ability to modify the migration of macrophages (1). The molecule responsible for the biological actions ascribed to MIF was identified and cloned in 1989 (2). Initially found to activate macrophages at inflammatory sites, it has been shown to possess pluripotential actions in the immune system. MIF has been shown to be expressed in human diseases which include inflammation, injury, ischaemia or malignancy. MIF also has a unique relationship with glucocorticoids by overriding their anti-inflammatory effects.

Recent studies have indicated that monoclonal antibody antagonism of MIF may be useful in the treatment of sepsis, certain types of cancers and delayed type hypersensitivity. Antibody antagonism of MIF has also been shown to have activity in adjuvant- or collagen-induced arthritis animal models and other models of inflammatory and immune diseases.

Although antibody antagonism of MIF is one potential way to provide therapeutic treatments, such biological molecules can be expensive to prepare on a commercial basis and further, can be limited in the way they are administered (generally by injection) and do not readily lend themselves to formulations for administration by other means eg oral

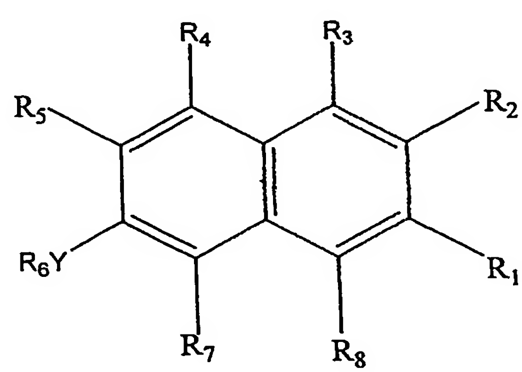
administration.

Small molecule inhibitors may overcome one or more such difficulties connected with the use of biological therapeutic treatments. There exists a need, therefore, for small molecule inhibitors of the cytokine or biological activity of MIF.

SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

In a first aspect, the present invention provides a method of inhibiting cytokine or biological activity of MIF comprising contacting MIF with a cytokine or biological activity inhibiting effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or prodrug thereof



wherein

Y is O, NR_9 or $S(O)_q$,

R_1 is selected from hydrogen, C_{1-6}alkyl, $-(CR_{10}R_{10'})_n$halo, $-(CR_{10}R_{10'})_nOR_{11}$, $-(CR_{10}R_{10'})_n-SR_{11}$, $-(CR_{10}R_{10'})_n-N(R_{12})_2$, $-(CR_{10}R_{10'})_nS(O)R_{11}$, $-(CR_{10}R_{10'})_nS(O)_2R_{11}$,

$-(CR_{10}R_{10'})_n-S(O)_3R_{11}$, $-(CR_{10}R_{10'})_nC(O)R_{13}$, $-(CR_{10}R_{10'})_n-C(=NR_{14})R_{15}$ or $-(CR_{10}R_{10'})_nR_{16}$;

R_2 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $-(CR_{10}R_{10'})_mOR_{17}$, $-(CR_{10}R_{10'})_mSR_{17}$, $-(CR_{10}R_{10'})_mNR_{18}R_{19}$, $-(CR_{10}R_{10'})_mS(O)R_{20}$, $-(CR_{10}R_{10'})_mS(O)_2R_{20}$, $-(CR_{10}R_{10'})_mC(O)R_{20}$, $-(CR_{10}R_{10'})_mC(S)R_{20}$, $-(CR_{10}R_{10'})_mC(=NR_{11})R_{15}$, $-(CR_{10}R_{10'})_mR_{16}$;

R_3, R_4 and R_5 are independently selected from H, C_{1-3}alkyl, $-(CR_{10}R_{10'})_nN(R_{14})_2$, $-(CR_{10}R_{10'})_nOR_{14}$, $-(CR_{10}R_{10'})_nSR_{14}$ and $-(CR_{10}R_{10'})_n$halo;

R_6 is selected from H, C_{1-6}alkyl, $-C(O)C_{1-6}$alkyl, $-(CR_{10}R_{10'})_nR_{21}$, or R_6Y and R_5 together may form $-X-(CH_2)_t-Z-$, where X and Z may be independently selected from O, S or NR_{14};

R_7 and R_8 are independently selected from H, C_{1-3}alkyl, C_{2-3}alkenyl, C_{2-3}alkynyl, $-(CR_{10}R_{10'})_nR_{22}$;

R_9 is selected from H or C_{1-6}alkyl;

Each R_{10} and $R_{10'}$ is independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, halogen, OR_{11}, SR_{11}, C_{1-3}alkoxy, CO_2R_{14}, $N(R_{14})_2$, -CN, NO_2, aryl or heterocyclyl;

R_{11} is hydrogen or C_{1-6}alkyl;

Each R_{12} is independently selected from hydrogen, C_{1-6}alkyl, $NH-C(=NR_{14})R_{15}$, $C(O)R_{14}$, $C(S)R_{14}$;

R_{13} is hydrogen, C_{1-6}alkyl, OR_{14}, SR_{14}, $N(R_{14})_2$;

Each R_{14} is independently selected from hydrogen or C_{1-3}alkyl;

R_{15} is C_{1-6}alkyl, NH_2, $NH(C_{1-3}$alkyl) or $N(C_{1-3}$alkyl$)_2$, OR_{23} or SR_{23};

R_{16} is hydroxy, C_{1-3}alkoxy, SH, SC_{1-3}alkyl, halo, $C(O)R_{32}$, $C(R_{24})_3$, CN, aryl and heterocyclyl;

R_{17} is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(CR_{26}R_{26'})_sR_{27}$, $C(O)R_{25}$, CO_2R_{25}, $C(S)R_{25}$, $C(S)OR_{25}$, $S(O)R_{25}$, $S(O)_2R_{25}$, $[C(O)CH(R_{29})NH]_r$-R_{23} and $[sugar]_r$;

R_{18} and R_{19} are independently selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(CR_{26}R_{26'})_sR_{27}$, $C(O)R_{25}$, $C(S)R_{25}$, $S(O)R_{25}$, $S(O)_2R_{25}$, $[C(O)CH(R_{29})NH]_r$-R_{23}, $[sugar]_r$ and NH-C(=N)NH_2;

R_{20} is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, OR_{28}, SR_{28}, $N(R_{28})_2$, $[NH\text{-}CHR_{29}C(O)]_r$-$OR_{23}$, $[sugar]_r$, and $(CR_{26}R_{26'})_sR_{27}$;

R_{21} is OR_{28}, SR_{28}, halo, $N(R_{25})_2$;

R_{22} is halo or $C(R_{24})_3$;

R_{23} is hydrogen or C_{1-3}alkyl;

Each R_{24} is independently selected from H, Cl or F;

Each R_{25} is independently selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, aryl, $(CR_{26}R_{26'})_sR_{27}$;

Each R_{26} and $R_{26'}$ is independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, halogen, hydroxy, C_{1-3}alkoxy, CO_2H, CO_2C_{1-3}alkyl, NH_2, $NH(C_{1-3}alkyl)$, $N(C_{1-3}alkyl)_2$, CN, NO_2, aryl or heteroaryl;

R_{27} is hydroxy, C_{1-3}alkoxy, SH, SC_{1-3}alkyl, halo, NH_2, $NH(C_{1-3}alkyl)$, $N(C_{1-3}alkyl)_2$,

$C(O)R_{32}$, aryl or heterocyclyl;

Each R_{28} is independently selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(CR_{26}R_{26'})_sR_{31}$;

R_{29} is the characterising group of an amino acid;

Each R_{30} and $R_{30'}$ is independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, hydroxy, C_{1-3}alkoxy, halogen, NH_2, $NH(C_{1-3}alkyl)$, $N(C_{1-3}alkyl)_2$, CO_2H, CO_2C_{1-3}alkyl, CN, NO_2, aryl or heterocyclyl;

R_{31} is halogen, hydroxy, C_{1-3}alkoxy, NH_2, $NH(C_{1-3}alkyl)$, $N(C_{1-3}alkyl)_2$, $C(O)R_{32}$, aryl or heterocyclyl;

R_{32} is C_{1-3}alkyl, OH, C_{1-3}alkoxy, aryl, aryloxy, heterocyclyl or heterocyclyloxy;

q is 0, 1, 2 or 3;
n is 0, 1, 2 or 3;
m is 0 or 1 to 20;
r is 1 to 5;
s is 1 to 10; and
t is 1 or 2;

wherein an alkyl, alkenyl, alkynyl, alkyloxy, aryl or heterocyclyl group may be optionally substituted one or more times.

In another aspect, the invention provides a method of treating, preventing or diagnosing a disease or condition wherein MIF cytokine or biological activity is implicated comprising the administration of a treatment, prevention or diagnostic effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.

In a further aspect, there is provided the use of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof in the manufacture of a medicament for the treatment, prevention or diagnosis of a disease or condition wherein MIF cytokine or biological activity is implicated.

In particular, the invention provides a method of treating, diagnosing or preventing autoimmune diseases, solid or haemopoitic tumours, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising:

Rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis) spondyloarthropathies (including but not limited to ankylosing spondylitis, reactive arthritis, Reiter's syndrome), crystal arthropathies (including but not limited to gout, pseudogout, calcium pyrophosphate deposition disease), Lyme disease, connective tissue diseases (including but not limited to systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, Sjögren's syndrome), vasculitides (including but not limited to polyarteritis nodosa, Wegener's granulomatosis, Churg-Strauss syndrome), glomerulonephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), peptic ulceration, gastritis, oesophagitis, liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to diabetes mellitus, thyroiditis, myasthenia gravis, sclerosing cholangitis, primary biliary cirrhosis), pulmonary diseases (including but not limited to diffuse interstitial lung diseases, pneumoconioses, fibrosing alveolitis, asthma, bronchitis, bronchiostatis, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to colon cancer, lymphoma, lung cancer, melanoma, prostate cancer, breast cancer, stomach cancer, leukemia, cervical cancer and metastatic cancer), atherosclerosis (eg ischaemic heart disease, myocardial infarction, stroke, peripheral vascular disease), disorders of the hypothalamic-pituitary-adrenal axis, brain disorders (eg Alzheimers, multiple sclerosis), corneal disease, iritis, iridocyclitis, cataracts, uveitis, sarcoidosis, diseases characterised by modified angiogenesis (eg diabetic

retinopathy, rheumatoid arthritis, cancer), endometrial function (menstruation, implantation, endometriosis), psoriasis, endotoxic (septic) shock, exotoxic (septic) shock, infective (true septic) shock, other complications of infection, pelvic inflammatory disease, transplant rejection, allergies, allergic rhinitis, bone diseases (eg osteoporosis, Paget's disease), atopic dermatitis, UV(B)-induced dermal cell activation (eg sunburn, skin cancer), malarial complications, diabetes mellitus, pain, inflammatory consequences of trauma or ischaemia, testicular dysfunctions, and wound healing,

comprising the administration of a treatment, diagnosis or prevention effective amount of a compound of Formula (I) to a subject in need thereof.

A further aspect of the invention provides for the use of a compound of Formula (I) in the manufacture of a medicament for the treatment of a disease or condition as above.

The compounds of formula (I) may also have an inhibitory effect on the tautomerase activity also associated with MIF. This may form a further aspect of the invention.

In preferred embodiments, the compounds of Formula (I) are used to treat or prevent a disease or condition, particularly in a human subject.

Certain compounds of Formula (I) are novel, and these form a further aspect of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 graphically depicts the effect of a 1M ratio equivalent of 6,7-dimethoxy-2-naphthanoic acid on MIF-induced proliferation of human dermal fibroblasts.

Figure 2 graphically depicts the effect of a 1M ratio equivalent of 6-hydroxy-2-naphthalene-sulfonic acid on MIF-induced proliferation of human dermal fibroblasts.

Figure 3 graphically depicts the effect of different doses of 6,7-dihydroxynaphthalene-3-sulfonic acid on IL-1 induced COX-2 expression.

Figure 4 graphically depicts the effect of a combination of dexamethasone and 6,7-dihydroxynaphthalene-3-sulfonic acid on IL-1 induced COX-2 expression.

Figure 5 graphically depicts the arthritis index in the rat adjuvant-induced arthritis model for 6,7-dimethoxy-2-naphthanoic acid.

Figure 6 graphically depicts the synovial fluid cell number in the rat adjuvant-induced arthritis model for 6,7-dimethoxy-2-naphthanoic acid.

Figure 7 graphically depicts the effect of 6,7-dihydroxynaphthalene-3-sulfonic acid on *in vivo* serum IL-1 production in a murine endotoxic shock model.

Figure 8 graphically depicts the effect of 6,7-dihydroxynaphthalene-3-sulfonic acid on *in vivo* serum IL-6 production in a murine endotoxic shock model.

Figure 9 graphically depicts the toxicity effect of a number of compounds in Formula (I) on *in vitro* S112 cells.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "alkyl", either used alone or in compound terms such as NHCalkyl, $N(Calkyl)_2$ etc, refers to monovalent straight, branched or, where appropriate, cyclic aliphatic radicals having from 1 to 3, 1 to 6, 1 to 10 or 1 to 20 carbon atoms as appropriate, ie methyl, ethyl, n-propyl, iso-propyl, cyclopropyl, n-butyl, sec-butyl, t-butyl and cyclobutyl, n-pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, cyclopentyl, n-hexyl, 1- 2- 3- or 4- methylpentyl, 1- 2- or 3-ethylbutyl, 1 or 2- propylpropyl or cyclohexyl.

An alkyl group may be optionally substituted one or more times by halo (eg chloro, fluoro or bromo), CN, NO_2, CO_2H, CO_2C_{1-6}alkyl, OH, methyl, ethyl, propyl, butyl, methoxy, ethoxy, propoxy, butoxy, NH_2, NH(C_{1-6}alkyl) or NH(C_{1-6}alkyl)$_2$. A preferred optional substituent is a polar substituent. Examples of alkoxy include methoxy , ethoxy, *n*-propoxy, *iso*-propoxy, cyclopropoxy, and butoxy (*n*-, *sec*- *t*- and cyclo) pentoxy and hexyloxy. The "alkyl" portion of an alkoxy group may be substituted as described above.

As used herein, the term "alkenyl" refers to straight, branched or, where appropriate, cyclic carbon containing radicals having one or more double bonds between carbon atoms. Examples of such radicals include vinyl, allyl, butenyl, or longer carbon chains such as those derived from palmitoleic, oleic, linoleic, linolenic or arachidonic acids. An alkenyl group may be optionally substituted one or more times by halo (eg chloro, fluoro or bromo), CN, NO_2, CO_2H, CO_2C_{1-6}alkyl, OH, methyl, ethyl, propyl, butyl, methoxy, ethoxy, propoxy, butoxy, NH_2, NH(C_{1-6}alkyl) or NH(C_{1-6}alkyl)$_2$. A preferred optional substituent is a polar substituent.

As used herein, the term "alkynyl" refers to straight or branched carbon containing radicals having one or more triple bonds between carbon atoms. Examples of such radicals include propargyl, butynyl and hexynyl. An alkynyl group may be optionally substituted one or more times by halo (eg chloro, fluoro or bromo), CN, NO_2, CO_2H, CO_2C_{1-6}alkyl, OH, methyl, ethyl, propyl, butyl, methoxy, ethoxy, propoxy, butoxy, NH_2, NH(C_{1-6}alkyl) or NH(C_{1-6}alkyl)$_2$. A preferred optional substituent is a polar substituent.

Examples of suitable NH(alkyl) and N(alkyl)$_2$ include methylamino, ethylamino, *n*-propylamino, *iso*-propylamino, dimethylamino, diethylamino and di-isopropylamino.

The term "halogen" (or "halo") refers to fluorine (fluoro), chlorine (chloro), bromine (bromo) or iodine (iodo).

As used herein, "the characterising group of an amino acid" refers to the substituent at C_2 of an amino acid and which defines the amino acid. For example, methyl is the

characterising group of alanine, phenylmethyl is the characterising group of phenylalanine and hydroxymethyl is the characterising group of serine.

The term "sugar" refers to a pyranosyl or furanosyl moiety such as derived from glucose, galactose, mannose, allose, altrose, gluose, idose, talose, ribose, arabinose or xylose.

An aryl group refers to a C_6-C_{12} aromatic carbocycle, for example, phenyl or naphthyl. An aryl group, either alone or part of a phenoxy, benzyl or benzyloxy group may be optionally substituted one or more times by halo (eg, chloro, fluoro or bromo), CN, NO_2, CO_2H, CO_2C_{1-6}alkyl, OH, methyl, ethyl, propyl, butyl, methoxy, ethoxy, propoxy or butoxy.

As used herein, the term "heterocyclyl" refers to a cyclic, aliphatic or aromatic radical containing at least one heteroatom independently selected from O, N or S. Examples of suitable heterocyclyl groups include furyl, pyridinyl, pyrimidinyl, pyrazolyl, piperidinyl, pyrrolyl, thyaphenyl, oxazolyl, imidazolyl, thiazolyl, isoxazolyl, isothiazolyl, quinolyl, isoquinolyl, indolyl, benzofuranyl, benzothiophenyl, triazolyl, terazolyl, oxadiazolyl and purinyl. A heterocyclyl group may be optionally substituted one or more times by halo (eg, chloro, fluoro or bromo), CN, NO_2, CO_2H, CO_2C_{1-6}alkyl, OH, methyl, ethyl, propyl, butyl, methoxy, ethoxy, propoxy or butoxy.

In a preferred embodiment, one or more of the following definitions apply:

Y is O, NH, NC_{1-6}alkyl, or $S(O)_q$ wherein q is 0, 1, 2 or 3;

R_1 is hydrogen, C_{1-6}alkyl, $(CH_2)_nOH$, $(CH_2)_nNH_2$, $(CH_2)_nSH$, $(CH_2)_nCF_3$, $(CH_2)_nCO_2H$, $(CH_2)_nCO_2C_{1-3}$alkyl, $(CH_2)_nC(O)NH_2$, $(CH_2)_nC(O)NHC_{1-3}$alkyl, $(CH_2)_nC(O)N(C_{1-3}alkyl)_2$, $(CH_2)_nSO_3H$, $(CH_2)_nSO_3C_{1-3}$alkyl, where n is 0, 1, 2 or 3;

R_2 is selected from C_{1-20}alkyl, C_{1-20}alkenyl, $(CR_{10}R_{10'})_mOH$, $(CR_{10}R_{10'})_mOC_{1-20}$alkyl, $(CR_{10}R_{10'})_mOC_{1-20}$alkenyl, $(CR_{10}R_{10'})_mOC(O)C_{1-20}$alkyl, $(CR_{10}R_{10'})_mOC(O)C_{1-20}$alkenyl, $(CR_{10}R_{10'})_mOC(O)$aryl, $(CR_{10}R_{10'})_mO[C(O)CH(R_{29})NH]_r$-H, $(CR_{10}R_{10'})_mO[sugar]_r$,

$(CR_{10}R_{10'})_mNHC_{1-20}$alkyl, $(CR_{10}R_{10'})_mN(C_{1-20}alkyl)_2$, $(CR_{10}R_{10'})_mNHC_{1-20}$alkenyl, $(CR_{10}R_{10'})_mN(C_{1-20}alkenyl)_2$, $(CR_{10}R_{10'})_mNHC(O)C_{1-20}$alkyl, $(CR_{10}R_{10'})_mNHC(O)C_{1-20}$alkenyl, $(CR_{10}R_{10'})_mNHC(O)$aryl, $(CR_{10}R_{10'})_mNH[C(O)CH(R_{29})NH]_r$-H, $(CR_{10}R_{10'})_mNH$-[sugar]$_r$, $(CR_{10}R_{10'})_mSO_3H$, $(CR_{10}R_{10'})_mSO_3C_{1-20}$alkyl, $(CR_{10}R_{10'})_mSO_3C_{1-20}$alkenyl, $(CR_{10}R_{10'})_mC(O)C_{1-20}$alkyl, $(CR_{10}R_{10'})_mC(O)C_{1-20}$alkenyl, $(CR_{10}R_{10'})_mCO_2H$, $(CR_{10}R_{10'})_mCO_2C_{1-20}$alkyl, $(CR_{10}R_{10'})_mCO_2C_{1-20}$alkenyl, $(CR_{10}R_{10'})_mC(O)NHC_{1-20}$alkyl, $(CR_{10}R_{10'})_mC(O)N(C_{1-20}alkyl)_2$, $(CR_{10}R_{10'})_mC(O)NHC_{1-20}$alkenyl, $(CR_{10}R_{10'})_mC(O)N(C_{1-20}alkenyl)_2$, $(CR_{10}R_{10'})_mC(O)[NHCH(R_{29})C(O)]_r$-OH, $(CR_{10}R_{10'})_mC(O)$[sugar]$_r$; wherein each R_{10} and $R_{10'}$ is independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, halogen, OH, OC_{1-6}alkyl, CO_2H, CO_2C_{1-3}alkyl, NH_2, NHC_{1-3}alkyl, $-N(C_{1-3}alkyl)_2$, CN, NO_2, aryl or heterocyclyl; R_{29} is the characterising group of an amino acid, m is 0 or an integer from 1 to 20 and r is an integer from 1 to 5;

R_3, R_4 and R_5 are independently selected from hydrogen, halogen, amino, OH or SH;

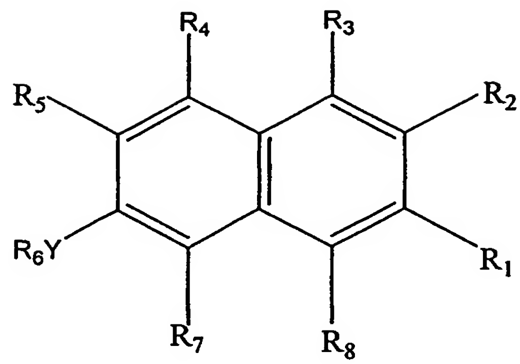
R_7 and R_8 are independently selected from hydrogen, C_{1-3}alkyl, or $(CH_2)_nR_{22}$, wherein R_{22} is halo, CH_2halo, $CH(halo)_2$ or $C(halo)_3$ and n is 0, 1, 2 or 3;

At least one of R_{10} and $R_{10'}$ is hydrogen;

At least one of R_{26} and $R_{26'}$ is hydrogen;

At least one of R_{30} and $R_{30'}$ is hydrogen.

In certain preferred forms of the invention, the compounds of Formula (I) comprise:



wherein

Y is O, NR_9 or $S(O)_q$;

R_1 is hydrogen, C_{1-6}alkyl, $-(CH_2)_nC(O)R_{13}$, $-(CH_2)_nS(O)_3R_{11}$, $-(CH_2)_nNH_2$, $-(CH_2)_nOH$, $-(CH_2)_nSH$ and $-(CH_2)_nCF_3$, where R_{11} and R_{13} are defined above;

R_2 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $-(CR_{10}R_{10'})_mOR_{17}$, $-(CR_{10}R_{10'})_mSR_{17}$, $-(CR_{10}R_{10'})_mNR_{18}R_{19}$, $-(CR_{10}R_{10'})_mS(O)R_{20}$, $-(CR_{10}R_{10'})_mS(O)_2R_{20}$, $-(CR_{10}R_{10'})_mC(O)R_{20}$, $-(CR_{10}R_{10'})_mC(S)R_{20}$, $-(CR_{10}R_{10'})_mC(=NR_{11})R_{15}$, $-(CR_{10}R_{10'})_mR_{16}$, where m, R_{10}, $R_{10'}$, R_{11}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20} are as defined above;

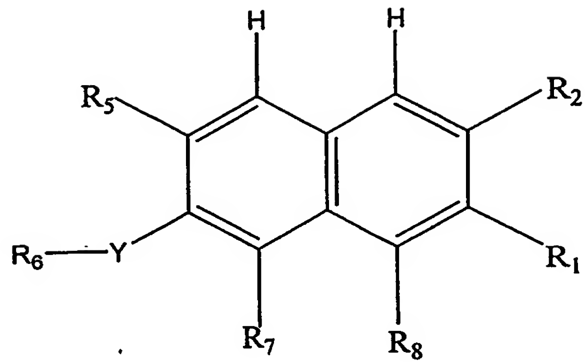
R_3, R_4 and R_5 are independently selected from hydrogen, halo, amino, OH or SH;

R_6 is hydrogen, C_{1-3}alkyl, CH_2halo, CH_2OH, CH_2SH;

R_7 and R_8 are hydrogen, C_{1-3}alkyl, $(CH_2)_nR_{22}$, where R_{22} is defined above, and

q and n are 0, 1, 2 or 3.

More preferably, the compounds of formula (I) comprise:



wherein

Y is O, NR_9 or $S(O)_q$;

R_1 is hydrogen, $(CH_2)_nCO_2H$, $(CH_2)_nSO_3H$, $(CH_2)_nNH_2$, C_{1-3}alkyl, $(CH_2)_nOH$ and $(CH_2)_nCF_3$;

R_2 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $-(CR_{10}R_{10'})_mOR_{17}$, $-(CR_{10}R_{10'})_mSR_{17}$, $-(CR_{10}R_{10'})_mNR_{18}R_{19}$, $-(CR_{10}R_{10'})_mS(O)R_{20}$, $-(CR_{10}R_{10'})_mS(O)_2R_{20}$, $-(CR_{10}R_{10'})_mC(O)R_{20}$, $-(CR_{10}R_{10'})_mC(S)R_{20}$, $-(CR_{10}R_{10'})_mC(=NR_{11})R_{15}$, $-(CR_{10}R_{10'})_mR_{16}$, where m, R_{10}, $R_{10'}$, R_{11}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20} are as defined above;

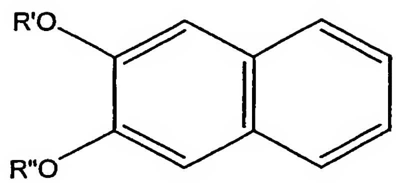
R_5 is hydrogen, OH and OCH_3;

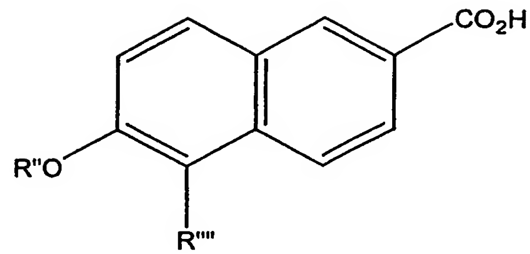
R_6 is hydrogen, C_{1-3}alkyl, CH_2halo, CH_2OH, CH_2SH;

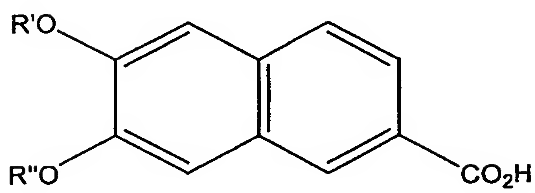
R_7 and R_8 are independently hydrogen, CH_3, CF_3 or CCl_3;

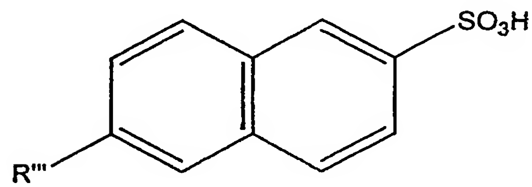
and q and n are 0, 1, 2 or 3.

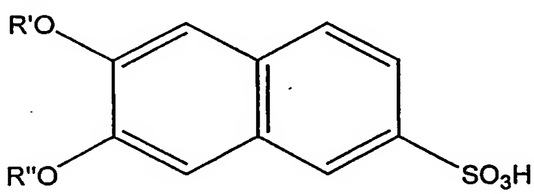
Examples of suitable compounds for use in the invention may include:

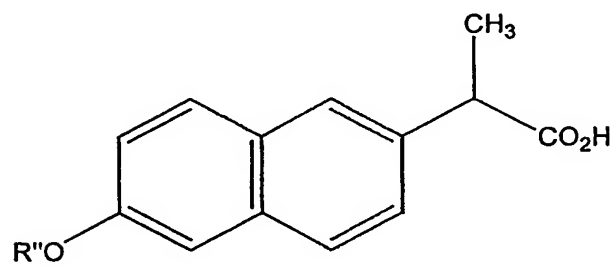












where R′ is H or C_{1-3}alkyl;

R′′ is H or C_{1-3}alkyl;

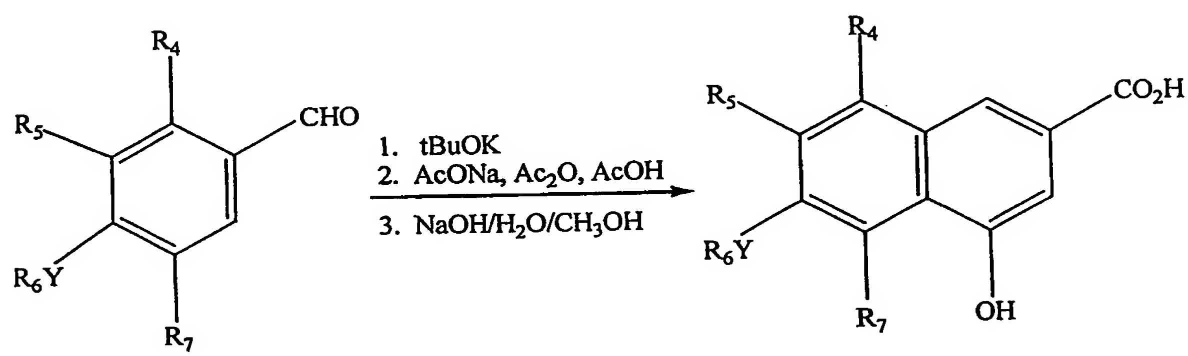
R′′′ is OH or SO_3H; and

R′′′′ is H, SO_3H or NO_2.

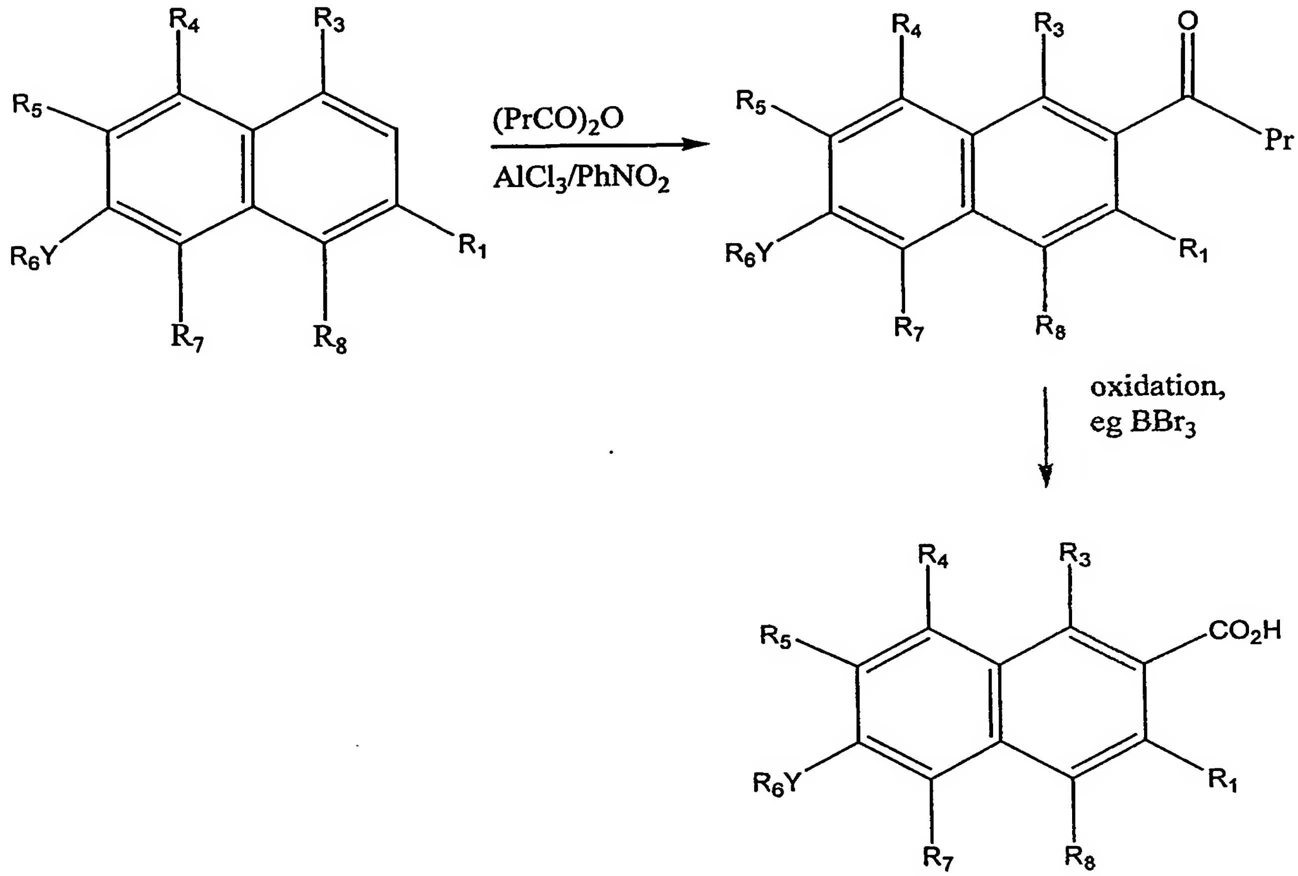
Compounds of Formula (I) may be prepared using the methods depicted or described herein or known in the art for example (3). It will be understood that minor modifications to methods described herein or known in the art may be required to synthesize particular compounds of Formula (I). General synthetic procedures applicable to the synthesis of compounds may be found in standard references such as *Comprehensive Organic Transformations*, R. C. Larock, 1989, VCH Publishers and *Advanced Organic Chemistry*, J. March, 4th Edition (1992), Wiley InterScience, and references therein, and may include Friedel Crafts acylation and/or electrophilic aromatic substitution of the naphthalene nucleus followed, where appropriate, by synthetic conversion (using standard procedures)

to the desired groups. It will also be recognised that certain reactive groups may require protection and deprotection during the synthetic process. Suitable protecting and deprotecting methods for reactive functional groups are known in the art for example in *Protective Groups in Organic Synthesis*, T. W. Green & P. Wutz, John Wiley & Son, 3rd Edition, 1999.

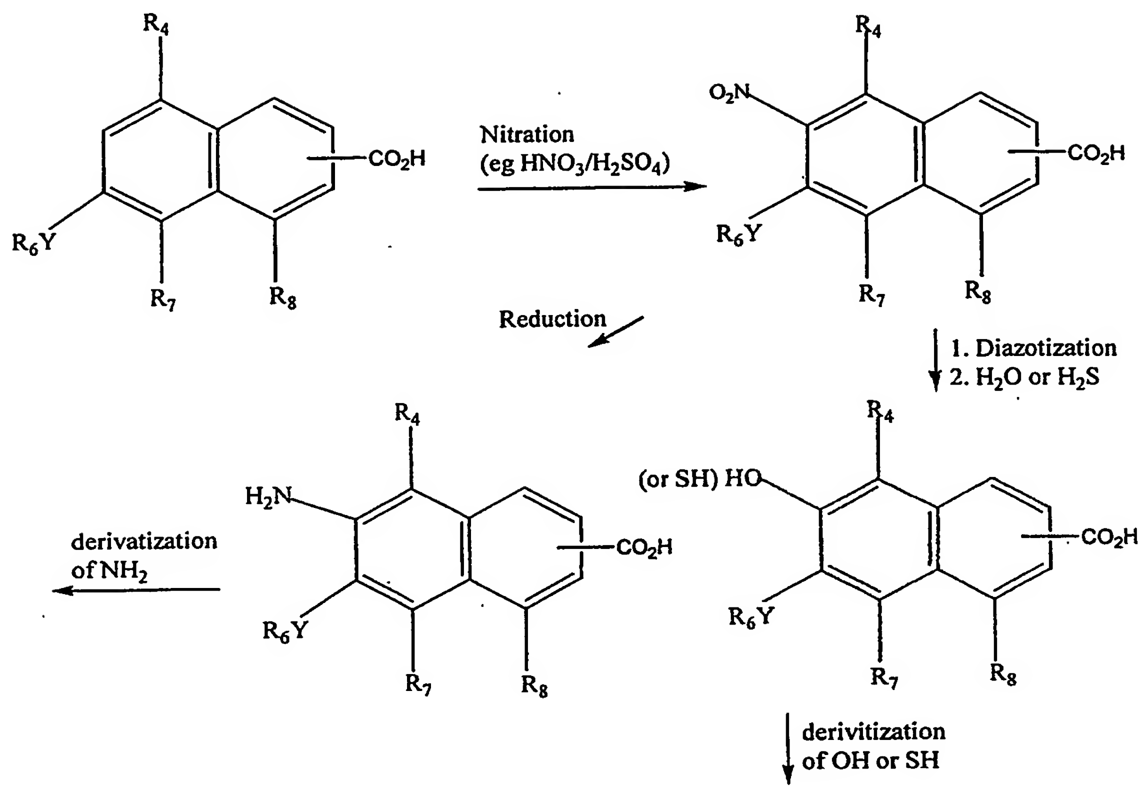
Thus, for certain embodiments of the invention, compounds of Formula (I), where R_1 or R_2 is CO_2H, can be prepared in accordance with the exemplified general methods or steps depicted in any of Schemes 1-3. Suitable starting materials can be obtained commercially or prepared using methods known in the art. Methodology relating to Schemes 1 and 2 can be found in (4) and (5) respectively. Methods for derivatizing NH_2, SH and OH to provide further compounds of Formula I are known in the art.



Scheme 1


R4
R3
R5
R6Y
R7
R8
R1
(PrCO)2O
AlCl3/PhNO2
O
Pr
oxidation,
eg BBr3
CO2H

Scheme 2



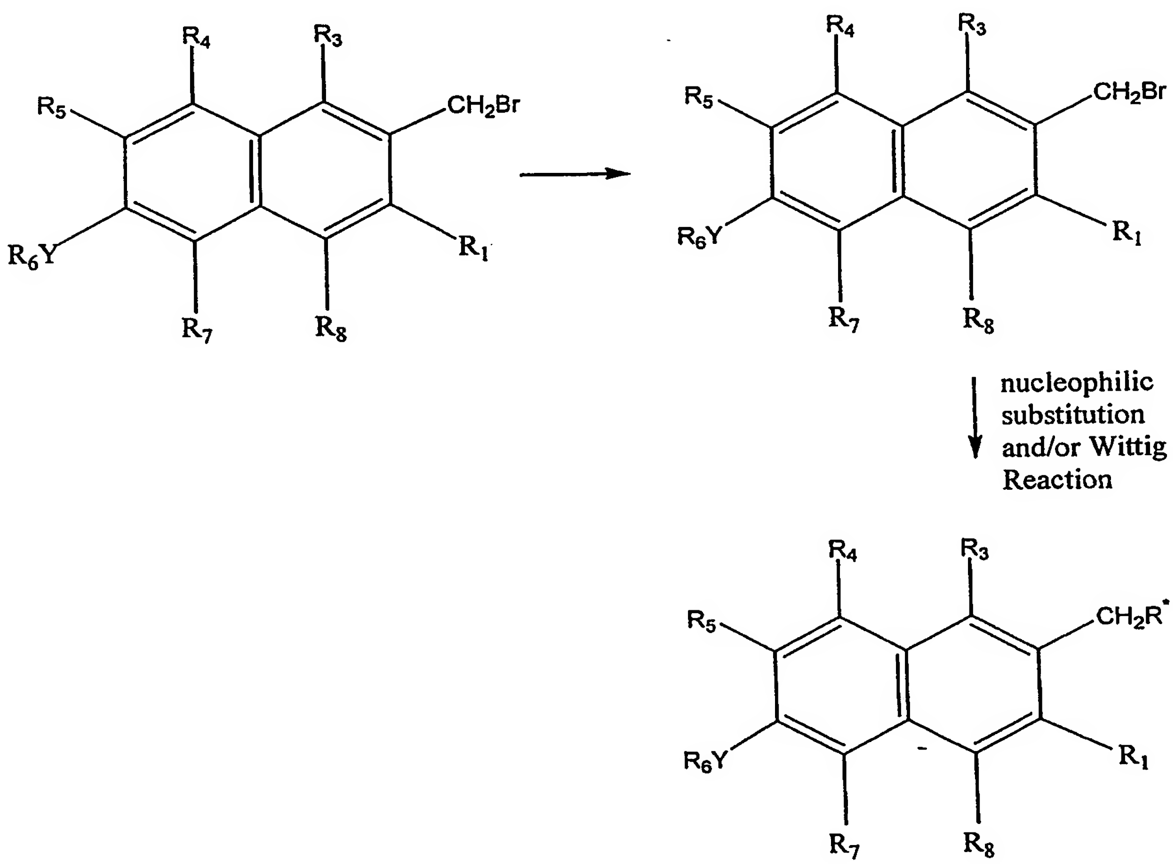
Scheme 3

Conversion of a CO_2H group to the amide ($CONH_2$) can be carried out using standard procedures in the art. Conversion of the amide to $C{=}NH(NH_2)$ can be achieved by aminolysis eg NH_3/dry methanol.

A methylene group can be inserted between the naphthalene nucleus and the carboxylic acid group by Arndt-Eistert synthesis, eg by conversion of the carboxylic acid to an acyl halide and conversion to the diazoketone. Rearrangement of the diazoketone (eg with silver oxide and water) affords access to the CH_2-CO_2H group. Repeating these steps allows for further incorporation of methylene groups. The CO_2H group can be converted as above.

In other embodiments, compounds of Formula (I), where R_1 or R_2 is a substituted methyl group, can be prepared by conversion of R_1 or R_2 being a methyl substituent into a halomethyl substituent (eg by treatment with a N-halosuccinimide such as NBS) followed

by nucleophilic substitution by an appropriate nucleophile and/or insertion of additional methylene groups by, for example, Wittig reaction (see Scheme 4 where R^* can be $(CH_2)_mOH$, $(CH_2)_mSH$, $(CH_2)_mNH_2$ $(CH_2)_mC(O)C_{1-6}alkyl$, $(CH_2)_mOC(O)C_{1-6}alkyl$, $(CH_2)_mOC_{1-6}alkyl$, $(CH_2)_mOphenyl$, $(CH_2)_mObenzyl$, $(CH_2)_mNHC_{1-6}alkyl$, $(CH_2)_mN(C_{1-6}alkyl)_2$, $(CH_2)_mNHphenyl$, $(CH_2)_mNHbenzyl$, $(CH_2)_mSC_{1-6}alkyl$, $(CH_2)_mSC(O)C_{1-6}alkyl$, $(CH_2)_mSphenyl$, $(CH_2)_mSbenzyl$, $(CH_2)_mNHsugar$, $(CH_2)_mSsugar$, $(CH_2)_mOsugar$, $(CH_2)_mNHC(O)C_{1-6}alkyl$, $(CH_2)_mNHC(O)phenyl$, $(CH_2)_mNHC(O)benzyl$, $(CH_2)_mNHCO_2C_{1-6}alkyl$, $(CH_2)_mNHCO_2phenyl$, or $(CH_2)_mNHCO_2benzyl$, where m is 0 or 1 to 20).

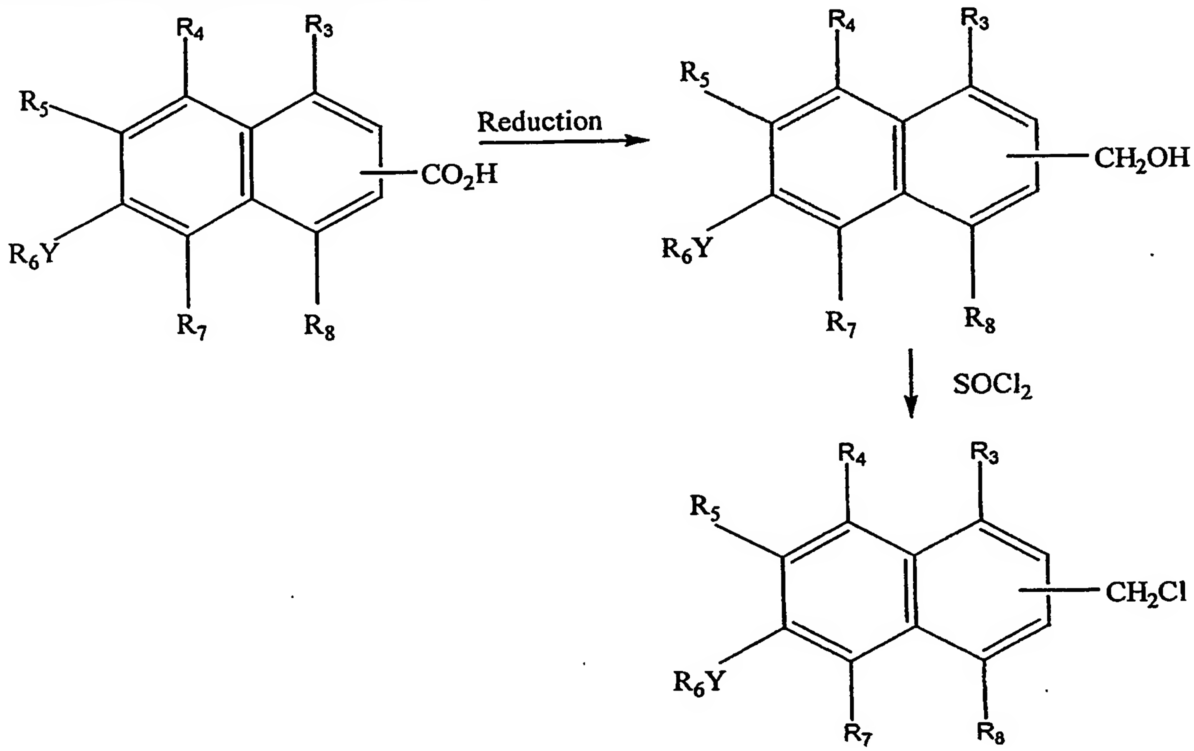


Scheme 4

In other embodiments, compounds where an O, S or N atom is directly bonded to the naphthalene nucleus can be prepared by suitable substitution (derivatization) of the

corresponding OH, SH or NH_2 group on the naphthalene nucleus eg by standard alkylating or acylating methodology.

In other embodiments, compounds where R_1 or R_2 is CH_2halo can be prepared by reaction of a suitable naphthalene carboxylic acid derivative with a reducing agent such as $LiAlH_4$, followed by halogenation, eg treatment with thionyl chloride.



Scheme 5

Coupling of compounds wherein R_1 or R_2 is CH_2halo with a C_{1-6}alkylhalide, halo$(CH_2)_{n/m}$heterocyclyl in the presence of CuLi affords the corresponding compounds where the R_1 and/or R_2 substituent is C_{1-6}alkyl, $(CH_2)_{n/m}$heterocyclyl.

Reaction of CH_2halo with NH_2-NH-C(=NH)-NH_2 in the presence of base affords access to compounds wherein R_1/R_2 is CH_2-NH-NH-C(=NH)-NH_2. Alternatively, reaction of the CH_2halo group with halo$(CH_2)_n$NH-NH-C(=NH)-NH_2 (where n is 1 or 2), affords the group $(CH_2)_n$NH-NH-C(=NH)-NH_2 where n is 2 or 3.

The term "salt, or prodrug" includes any pharmaceutically acceptable salt, ester, solvate, hydrate or any other compound which, upon administration to the recipient is capable of providing (directly or indirectly) a compound of Formula (I) as described herein. The term "pro-drug" is used in its broadest sense and encompasses those derivatives that are converted *in vivo* to the compounds of the invention. Such derivatives would readily occur to those skilled in the art, and include, for example, compounds where a free hydroxy group is converted into an ester, such as an acetate, or where a free amino group is converted into an amide. Procedures for acylating hydroxy or amino groups of the compounds of the invention are well known in the art and may include treatment of the compound with an appropriate carboxylic acid, anhydride or acylchloride in the presence of a suitable catalyst or base.

Suitable pharmaceutically acceptable salts include, but are not limited to, salts of pharmaceutically acceptable inorganic acids such as hydrochloric, sulphuric, phosphoric, nitric, carbonic, boric, sulfamic, and hydrobromic acids, or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, maleic, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, toluenesulphonic, benezenesulphonic, salicyclic sulphanilic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

Base salts include, but are not limited to, those formed with pharmaceutically acceptable cations, such as sodium, potassium, lithium, calcium, magnesium, ammonium and alkylammonium.

Basic nitrogen-containing groups may be quarternised with such agents as lower alkyl halide, such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates like dimethyl and diethyl sulfate; and others.

It will also be recognised that some compounds of formula (I) may possess asymmetric centres and are therefore capable of existing in more than one stereoisomeric form. The

invention thus also relates to compounds in substantially pure isomeric form at one or more asymmetric centres eg., greater than about 90% ee, such as about 95% or 97% ee or greater than 99% ee, as well as mixtures, including racemic mixtures, thereof. Such isomers may be prepared by asymmetric synthesis, for example using chiral intermediates, or by chiral resolution.

As used herein, MIF includes human or other animal MIF and derivatives and naturally occurring variants thereof which at least partially retain MIF cytokine or biological activity. Thus, the subject to be treated may be human or other animal such as a mammal. Non-human subjects include, but are not limited to primates, livestock animals (eg sheep, cows, horses, pigs, goats), domestic animals (eg dogs, cats), birds and laboratory test animals (eg mice rats, guinea pigs, rabbits). MIF is also expressed in plants (thus "MIF" may also refer to plant MIF) and where appropriate, compounds of Formula (I) may be used in botanical/agricultural applications such as crop control.

Reference herein to "cytokine or biological activity" of MIF includes the cytokine or biological effect on cellular function via autocrine, endocrine, paracrine, cytokine, hormone or growth factor activity, or via intracellular effects.

As used herein, the term "effective amount" relates to an amount of compound which, when administered according to a desired dosing regimen, provides the desired MIF cytokine inhibiting or treatment or therapeutic activity, or disease/condition prevention. Dosing may occur at intervals of minutes, hours, days, weeks, months or years or continuously over any one of these periods. A cytokine or biological activity inhibiting amount is an amount which will at least partially inhibit the cytokine or biological activity of MIF. A therapeutic, or treatment, effective amount is an amount of the compound which, when administered according to a desired dosing regimen, is sufficient to at least partially attain the desired therapeutic effect, or delay the onset of, or inhibit the progression of or halt or partially or fully reverse the onset or progression of a particular disease condition being treated. A prevention effective amount is an amount of compound which when administered according to the desired dosing regimen is sufficient to at least

partially prevent or delay the onset of a particular disease or condition. A diagnostic effective amount of compound is an amount sufficient to bind to MIF to enable detection of the MIF-compound complex such that diagnosis of a disease or condition is possible.

Suitable dosages may lie within the range of about 0.1 ng per kg of body weight to 1 g per kg of body weight per dosage. The dosage is preferably in the range of 1 μg to 1 g per kg of body weight per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage is in the range of 1 mg to 500 mg per kg of body weight per dosage. In another embodiment, the dosage is in the range of 1 mg to 250 mg per kg of body weight per dosage. In yet another preferred embodiment, the dosage is in the range of 1 mg to 100 mg per kg of body weight per dosage, such as up to 50 mg per kg of body weight per dosage. In yet another embodiment, the dosage is in the range of 1μg to 1mg per kg of body weight per dosage.

Suitable dosage amounts and dosing regimens can be determined by the attending physician or veterinarian and may depend on the desired level of inhibiting activity, the particular condition being treated, the severity of the condition as well as the general age, health and weight of the subject.

The active ingredient may be administered in a single dose or a series of doses. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a composition, preferably as a pharmaceutical composition.

The formulation of such compositions is well known to those skilled in the art. The composition may contain pharmaceutically acceptable additives such as carriers, diluents or excipients. These include, where appropriate, all conventional solvents, dispersion agents, fillers, solid carriers, coating agents, antifungal and antibacterial agents, dermal penetration agents, surfactants, isotonic and absorption agents and the like. It will be understood that the compositions of the invention may also include other supplementary physiologically active agents.

The carrier must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the composition and not injurious to the subject. Compositions include those suitable for oral, rectal, inhalational, nasal, transdermal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intraspinal, intravenous and intradermal) administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Depending on the disease or condition to be treated, it may or may not be desirable for a compound of Formula (I) to cross the blood/brain barrier. Thus the compositions for use in the present invention may be formulated to be water or lipid soluble.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (eg inert diluent, preservative, disintegrant (eg. sodium starch glycolate, cross-linked polyvinyl pyrrolidone, cross-linked sodium carboxymethyl cellulose)) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to

provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Compositions suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured base, usually sucrose and acacia or tragacanth gum; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia gum; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

The compounds of Formula (I) may also be administered intranasally or via inhalation, for example by atomiser, aerosol or nebulizer means.

Compositions suitable for topical administration to the skin may comprise the compounds dissolved or suspended in any suitable carrier or base and may be in the form of lotions, gel, creams, pastes, ointments and the like. Suitable carriers include mineral oil, propylene glycol, polyoxyethylene, polyoxypropylene, emulsifying wax, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. Transdermal devices, such as patches, may also be used to administer the compounds of the invention.

Compositions for rectal administration may be presented as a suppository with a suitable carrier base comprising, for example, cocoa butter, gelatin, glycerin or polyethylene glycol.

Compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Compositions suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bactericides and solutes which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage compositions are those containing a daily dose or unit, daily sub-dose, as herein above described, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the active ingredients particularly mentioned above, the compositions of this invention may include other agents conventional in the art having regard to the type of composition in question, for example, those suitable for oral administration may include such further agents as binders, sweeteners, thickeners, flavouring agents, disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

It will be recognised that other therapeutically active agents such as anti-inflammatory (eg steroids such as glucocorticoids) or anti-cancer agents may be used in conjunction with a compound of Formula (I). Compounds of Formula (I) when administered in conjunction with other therapeutically active agents may exhibit an additive or synergistic effect. These may be administered simultaneously, either as a combined form (ie as a single composition containing the active agents) or as discrete dosages. Alternatively, the other therapeutically active agents may be administered sequentially or separately with the compounds of the invention. Thus, the invention also relates to kits and combinations, comprising a compound of Formula (I) and one or more other therapeutically active ingredients for use in the treatment of diseases or conditions described herein.

Examples of suitable glucocorticoids include but are not limited to prednisolone, prednisone, cortisone acetate, beclamethasone, fluticasone, hydrocortisone and dexamethasone. A person skilled in the art would be able to identify other suitable glucocorticoids that may benefit from being used in a combination treatment with a MIF antagonist.

Glucocorticoids may be administered in single, daily or divided doses or as a continuous infusion. When administered orally, intravenously, intramuscularly, intralesionally or intra-cavity (e.g. intra-articular, intrathecal, intra-thoracic), dosages are typically between 1 mg to 1000 mg, preferably 1 mg to 100 mg, more preferably 1 mg to 50 mg or 1 mg to 10 mg per dose. When administered topically or by inhalation as a single, daily or divided dose, dosages are typically 1 ng to 1 μg, 1 ng to 1 mg, or 1 pg to 1 μg.

In one preferred aspect of the invention, the compounds of Formula (I) may be administered together with, simultaneously or sequentially, glucocorticoids. In such a therapy, the amount of glucocorticoid required may be significantly reduced.

The compounds of the invention may also be presented for use in veterinary compositions. These may be prepared by any suitable means known in the art. Examples of such compositions include those adapted for:

(a) oral administration, external application (eg drenches including aqueous and non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pellets for admixture with feedstuffs, pastes for application to the tongue;

(b) parenteral administration, eg subcutaneous, intramuscular or intravenous injection as a sterile solution or suspension; and

(c) topical application eg creams, ointments, gels, lotions, etc.

By virtue of their ability to bind to or antagonise MIF, compounds of Formula (I) or salts or derivatives thereof may be used as laboratory or diagnostic or *in vivo* imaging reagents. Typically, for such use the compounds would be labelled in some way, for example, radio isotope, fluorescence or colorimetric labelling, or be chelator conjugated. In particular, compounds of Formula (I) could be used as part of an assay system for MIF or as controls in screens for identifying other inhibitors. Those skilled in the art are familiar with such screens and could readily establish such screens using compounds of Formula (I). Those skilled in the art will also be familiar with the use of chelate conjugated molecules for *in vivo* diagnostic imaging.

Unless the context indicates otherwise, reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within the spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

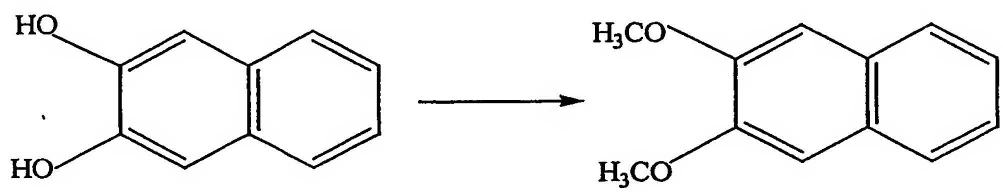
The invention will now be described with reference to the following examples which are included for the purpose of illustration only and are not intended to limit the generality of the invention hereinbefore described.

EXAMPLES

Synthesis of compounds of Formula (I).

Example 1: 6,7-Dimethoxy-2-naphthanoic acid

2,3-Dimethoxynaphthalene



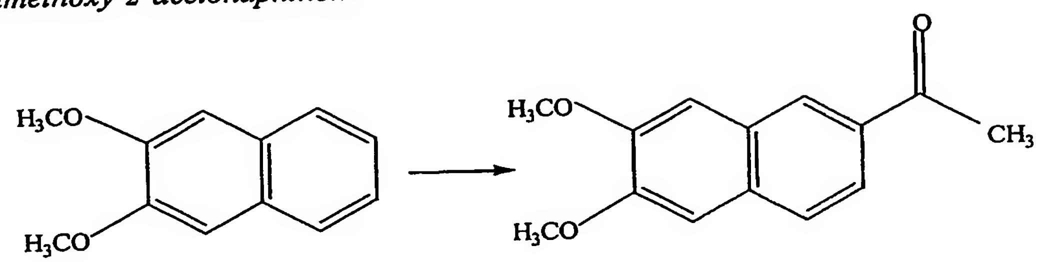
(1) (2)

A suspension of 2,3-dihydroxynaphthalene (5.00g, 0.0312 mol) in water (25 mL) in a three-necked round-bottomed flask was cooled in an ice-bath. Two pressure equilibrating funnels were set up and these charged with dimethyl sulphate (7.20 mL, 9.57 g, 0.0759 mol) and aqueous potassium hydroxide (5.57 g, 0.0993 mol in 17.0 mL of water) respectively. Both of these were added together dropwise over 10 minutes resulting in the suspension first dissolving and then a precipitate forming. The reaction was left overnight at room temperature. The solid was then filtered off, washed with water until the washings were neutral (5 x 200 mL), and dried to give 2,3-dimethoxynaphthalene (4.09 g, 70% yield) as a white powder;

R_f: 0.71 (19:1 $CHCl_3$:MeOH), 0.82 (9:1 $CHCl_3$:MeOH),
mp: 112-113 °C, lit.mp:113-116°C (Zee-Cheng et al J. Heterocylc. Chem., 1972, 9, 805-11);

^{1}H NMR ($CDCl_3$/TMS): δ 4.01 (s, 6 H, 2 x OCH_3), 7.13 (s, 2H), 7.33-7.36 (m, 2H), 7.68-7.71(m, 2H);
LRESI mass spectrum: *m/z* 189 (100%, MH^+).

6,7-Dimethoxy-2-acetonaphthone

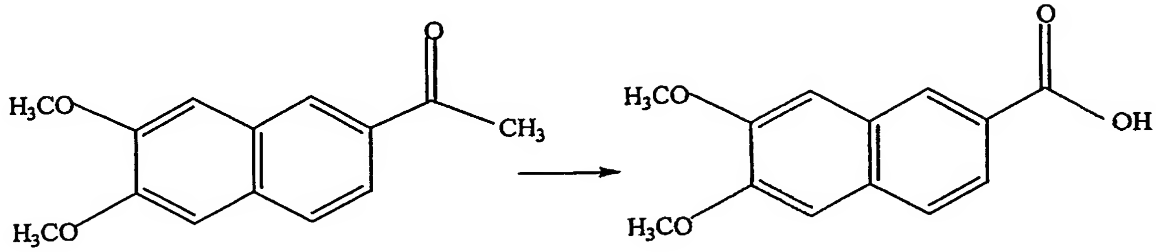


(2) (3)

A suspension of aluminium chloride (6.02g, 0.0451 mol) in sieve-dried nitrobenzene (10 mL) was cooled in an ice-bath and acetyl chloride (3.57 mL, 3.93 g, 0.0501 mol) added over 5 minutes. 2,3-Dimethoxynaphthalene (7.52 g, 0.0400 mol) in nitrobenzene (25 mL) was then added over 10 minutes. The reaction was stirred for a further 60 minutes at 0°C and then left overnight at room temperature. The mixture was poured onto a mixture of ice (60 g) and 10% HCl (100 mL). Chloroform (300 mL) was added and the two phases separated. The aqueous was further extracted with chloroform (2 x 150 mL) and the combined organics then washed with 5% aqueous sodium hydroxide (3 x 100 mL) and water (2 x 100 mL), dried (anhydrous Na_2SO_4), filtered and evaporated under vacuo to give a brown oil. This was flash column chromatographed (silica gel, chloroform) to give 6,7-dimethoxy-2-acetonaphthone (8.51 g. 93% yield) as an orange solid. A sample was further recrystallised from ethanol to give fine orange needles;

R_f: 0.36 ($CHCl_3$), 0.62 (25:1 $CHCl_3$:MeOH),
mp: 100-102°C, lit. mp: 113-116°C (Zee-Cheng et al supra);
^{1}H NMR ($CDCl_3$/TMS): δ 2.69 (s, 3H, $COCH_3$), 4.02 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 7.14 (s, 1H, H-8), 7.22 (s, 1H, H-5), 7.72 (d, 1H, $J_{4,3}$ 8.4 Hz, H-4), 7.89 (dd, 1H, $J_{3,1}$ 1.7 Hz, H-3), 8.33 (bs, 1H, H-1);
LRESI mass spectrum: *m/z* 231 (100%, MH^+).

6,7-Dimethoxy-2-naphthanoic acid



(3) (4)

Sodium hypochlorite (55 mL, 12.5% w/v) was first added to sodium hydroxide (1.80 g, 0.0450 mole) dissolved in water (5.5 mL). This solution was gently heated to 45°C and 6,7-dimethoxy-2-acetonaphthone (2.50 g. 0.0187 mole) then added. Heating was gradually increased until the suspension dissolved at a temperature of 85°C and the solution was maintained at 85°C for a further 60 minutes. The solution was then allowed to cool to room temperature and filtered to remove a small amount of orange gum. Small quantities of sodium bisulfite (spatulla ends) were then added to the filtrate until it no longer darkened iodine/starch indicator paper. The solution was then cooled in an ice-bath and concentrated Hcl added drop-wise until a pH of 1. The resultant white precipitate was filtered off, washed with cold water (3 x 20 mL) and dried under vacuum over a desiccant to give 6,7-dimethoxy-2-naphthanoic acid (2.2601 g, 90% yield as a white powder;

R_f: 0.36 (9:1 $CHCl_3$:MeOH),
mp: 248-250°C; lit. mp: 246-248°C (Zee-Cheng et al, *supra*)
^{1}H NMR ($CDCl_3$/CD_3OD/TMS): δ 4.02 (s, 3H, OCH_3), 4.03 (s, 3H,-OCH_3), 7.19 (s, 1H, H-8), 7.26 (s, 1H, H-5), 7.73 (d, 1H, $J_{4,3}$ 8.5 Hz, H-4), 7.93 (dd, 1H, $J_{3,1}$ 1.7 Hz, H-3), 8.47 (bs, 1H, H-1);
LRESI mass spectrum: *m/z* 233 (41%, MH^+), 255 (100%, MNa^+).

<u>*Example 2: 2-carboxy-6-hydroxynaphthalene-5-sulfonic acid*</u>

Conc. sulfuric acid (95-98%, 12 ml) was cooled in ice-bath and 6-hydroxy-2-naphthanoic acid (2.83 g; 15.05 mmol) added in small portions. The reaction mixture was stirred at room temperature for 4 hours. The white solid was filtered and recrystallised from water. This gave yield of 62%.

^{1}H NMR (DMSO-d6):δ 7.09 (d, 1H, J_{ortho}=8.7 Hz, aromatic), 7.90 (d, IH, J_{ortho}=9.3 Hz, aromatic), 7.95 (d, IH, J_{ortho}=8.7 Hz, aromatic), 8.41 (s, 1H, aromatic) and 8.66 (d, IH, J_{ortho}=9.1 Hz, aromatic).
Negative ion mass spectrum 267 *m/z* (100%).

Biological testing

6-Hydroxy-2-naphthalene-sulfonic acid was obtained commercially from Merck. 6,7-dihydroxynaphthalene-2-sulfonic acid (cat. No. 21, 896-0), S-(+)-6-methoxy-α-methyl-2-naphthalene acetic acid (cat. No. 25, 478-5) and 6-hydroxy-2-naphthanoic acid (cat. No. 46, 915-7) were obtained from Aldrich. 6,7-Dimethoxy-2-naphthanoic acid (4), 6,7-dimethoxynaphthalene and 2-carboxy-6-hydroxynaphthalene-5-sulfonic acid were prepared as described herein.

In vitro assay of MIF antagonism

The activity of each compound was studied in a bioassay utilising MIF-induced proliferation of human dermal fibroblasts. S112 human dermal fibroblasts were propagated in RPMI/10% foetal calf serum (FCS). Prior to experimentation, cells were seeded at 10^5 cells/ml in RPMI/0.1% BSA for 18 hours. At time point zero, culture medium was replaced with RPMI/10% FCS and treatments administered. Cells were treated with recombinant human macrophage migration inhibitory factor (MIF) 50 ng/ml (1.353x 10^{-9} M) and/or the compound at a 1 or 1000 molar ratio to the concentration of MIF. In some experiments the compound was combined with MIF at time point –30 minutes, prior to adding at time point zero. At time point 30 hours, cells were pulsed with 1 μCi^{3}H-thymidine. At time point 48 hours, cells were harvested using a semi-automated

cell harvester. The radioactivity incorporated into DNA was determined by liquid scintillation counting, with results expressed as [^{3}H] thymidine incorporation. The proliferation of untreated cells was expressed as 100% and the effect of MIF and each compound expressed in relative %.

The results for 6,7-dimethoxy-2-naphthanoic acid (4) and 6-hydroxy-2-naphthalene-sulfonic acid (5) are depicted on Figures 1 and 2 respectively.

Alternative in vitro assay of MIF antagonism

The activity of each compound was studied in a bioassay utilising MIF-dependent activation of human dermal fibroblasts. Sampey et al have shown that induction of the expression of cyclooxygenase-2 (COX-2) by the cytokine interleukin 1 (IL-1) is dependent upon the presence of MIF, i.e. can be prevented using specific anti-MIF monoclonal antibody (6). IL-1-induced COX-2 expression is therefore a MIF-dependent event.

S112 human dermal fibroblasts were propagated in RPMI/10% foetal calf serum (FCS). Prior to experimentation, cells were seeded at 10^5 cells/ml in RPMI/0.1% BSA for 18 hours. Cells were treated with recombinant human IL-1 (0.1 ng/ml) and with each compound at 1-100 μM. After 6 hours, cells were collected and intracellular COX-2 protein determined by permeabilisation flow cytometry. Cells permeabilised with 0.1% saponin were sequentially labelled with a mouse anti-human COX-2 monoclonal antibody and with sheep-anti-mouse F(ab)2 fragment labelled with fluoroscein isothiocyanate. Cellular fluorescence was determined using a flow cytometer. At least 5000 events were counted for each reading, each of which was performed in duplicate, and the results expressed in mean fluorescence intensity (MFI) after subtraction of negative control-labelled cell fluorescence.

The effect of each compound was determined by subtracting the IL-1+compound-treated cell MFI from the IL-1-treated cell MFI and expressed as % inhibition.

Results are shown in Table 1 below as % average inhibition of COX-2.

Table 1

	Compound	Concentration of compound	Average % inhibition	Number of experiments
1	6,7-dimethoxynaphthylene (2)	0.1 μM	-10.15	2
2	6-hydroxynaphthylene-2-sulfonic acid	50 μM	-25.03	2
3	6,7-dihydroxynaphthalene-2-sulfonic acid	50 μM	-25.4	9
4	2-carboxy-6-hydroxynaphthalene-5-sulfonic acid	0.1 μM	-23.7	1

Figure 3 shows a dose response curve for 6,7-dihydroxynaphthalene-2-sulphonic acid. This compound was tested for IL-1 induced COX-2 expression inhibition, as discussed above at a concentration of 0.01, 0.1, 1.0, 10 and 50 μM.

Effect of glucocorticoids on MIF antagonism

In vitro assay of MIF antagonism in the presence of glucocorticoid

The above alternative *in vitro* assay for analysing IL-1 induced COX-2 expression was repeated using 6,7-dihydroxynaphthalene-2-sulfonic acid (50 μM), dexamethasone (10^{-9} M) or a combination of dexamethasone (10^{-9} M) and 6,7-dihydroxynaphthalene-2-sulfonic acid (50 μM). The results are shown in Table 2 and Figure 4.

Table 2

Experiment	Compound	% inhibition
1	6,7-dihydroxynaphthalene-2-sulfonic acid	-38.0
2	dexamethasone	-63.8
3	6,7-dihydroxynaphthalene-2-sulfonic acid and dexamethasone	-83.3

In vivo assay of MIF antagonism

The activity of each compound was studied in the rat adjuvant-induced arthritis (AIA) model of rheumatoid arthritis. This model has been demonstrated to be dependent on MIF (6). Male Sprague-Dawley rats (150± 20 g) were used. Adjuvant arthritis was induced by intradermal injection at the tail base of 150 μ of a 10 mg/ml suspension of heat-inactivated *Mycobacterium tuberculosis* (Difco, Detroit, MI) in squalane. The compound was administered at a dose of 1.0 mg/kg body weight by once daily intraperitoneal injection on each day. Control animals received an identical volume injection of vehicle. Joint inflammation in adjuvant arthritis was assessed clinically as follows:

i) *Articular index/score*: A score of 0 (no observable erythema or swelling) to 4 (severe swelling and erythema) was given for each paw. All four paws were scored, resulting in a maximum possible score of 16 for each animal (7).

ii) *Synovial fluid cell number*: Joints were exposed by removal of overlying skin, needle arthrocentesis performed and joint space cells obtained by closed needle lavage with 2 ml saline using a 26 gauge needle and syringe. Lavaged cells from both ankle joints were pooled, washed in saline (300g for 5 minutes), and counted in a hemocytometer (Improved Nebauer, Weber, UK) (7).

The results for 6,7-dimethoxy-2-naphthanoic acid in relation to i) and ii) are depicted in Figures 5 and 6 respectively.

Alternative in vivo assay of MIF antagonism

The activity of 6,7-dihydroxynaphthalene-3-sulfonic acid was studied in the murine endotoxic shock model. This model has been previously shown to be dependent on MIF (8). Endotoxaemia was induced by intra-peritoneal injection of lipopolysaccharide (LPS) (15 mg/kg) in 400 µl saline. Mice were treated with a saline solution (control) only, a saline solution and LPS or 6,7-dihydroxynaphthalene-2-sulfonic acid at a dose of 15 mg/kg body weight by intra-peritoneal injection at 24 hours, 12 hours and 1 hour before intra-peritoneal LPS injection. After 24 hours mice were humanely killed by CO_2 inhalation then neck dislocation. Serum was obtained from blood obtained by cardiac puncture prior to death and measured for cytokines including interleukin 6 (IL-6) by ELISA. In certain experiments, serum was obtained 1.5 hours after LPS administration for the measurement of interleukin 1 (IL-1) or IL-6. The production of IL-1 and IL-6 has been previously shown to be dependent on MIF (9). Figure 7 shows analysis of serum IL-1 (ng/ml) when LPS is administered alone or in combination with 6,7-dihydroxynaphthalene-2-sulfonic acid. Figure 8 shows analysis of serum IL-6 (ng/ml) when LPS is administered alone or in combination with 6,7-dihydroxynaphthalene-2-sulfonic acid.

In vitro toxicity assay

The compounds of formula (I) may have low toxicity towards cells. The toxicity of compounds of formula (I) were examined *in vitro* to assess cytotoxicity. Human dermal fibroblast cell line (S112) cells were exposed to vehicle (control) or compounds of formula (I) (50 µM) in vehicle. Toxicity was assessed by analysis of apoptosis using flow cytometric detection of cell surface Annexin V binding and propidium iodide staining. At least 5000 events were analysed for each experiment. Cells positive for both Annexin V and propidium iodide were designated as apoptotic and cells negative for both Annexin V and propidium iodide were designated as viable. Results are expressed as the percentage (%) of cells with each of these labels. No compound of formula (I) induced apoptosis at levels above the control. The results for a number of compounds of formula (I) are shown in Figure 9.

Table 3: Key to compounds tested in Figure 9

Compound	Name
1	6,7-dihydroxynaphthalene-2-sulphonic acid
2	6,7-dimethoxynaphthalene
3	6,7-dimethoxy-2-naphthanoic acid
4	6,7-dihydroxynaphthalene
5	(S)-(+)-6-methoxy-α-methyl-2-naphthalene acetic acid
6	6-hydroxy-2-naphthanoic acid

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(3) *Tetrahedron*,1998 54(35), 10493-10511.

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(5) *J. Med. Chem.*, 1997, 40, 1186-1194.

(6) Sampey, A.V. et al, *Arthritis Rheum* 44:1273-1280, 2001.

(7) Leech. M., *et al., Arthritis Rheum.*, 41:910-917, 1998.

(8) Bernhagen, J. *et al., Nature*, 365, 1993 (1999), 756-759.

(9) Bozza, *et al., Exp. Med.* 189 (1999), 341-346.

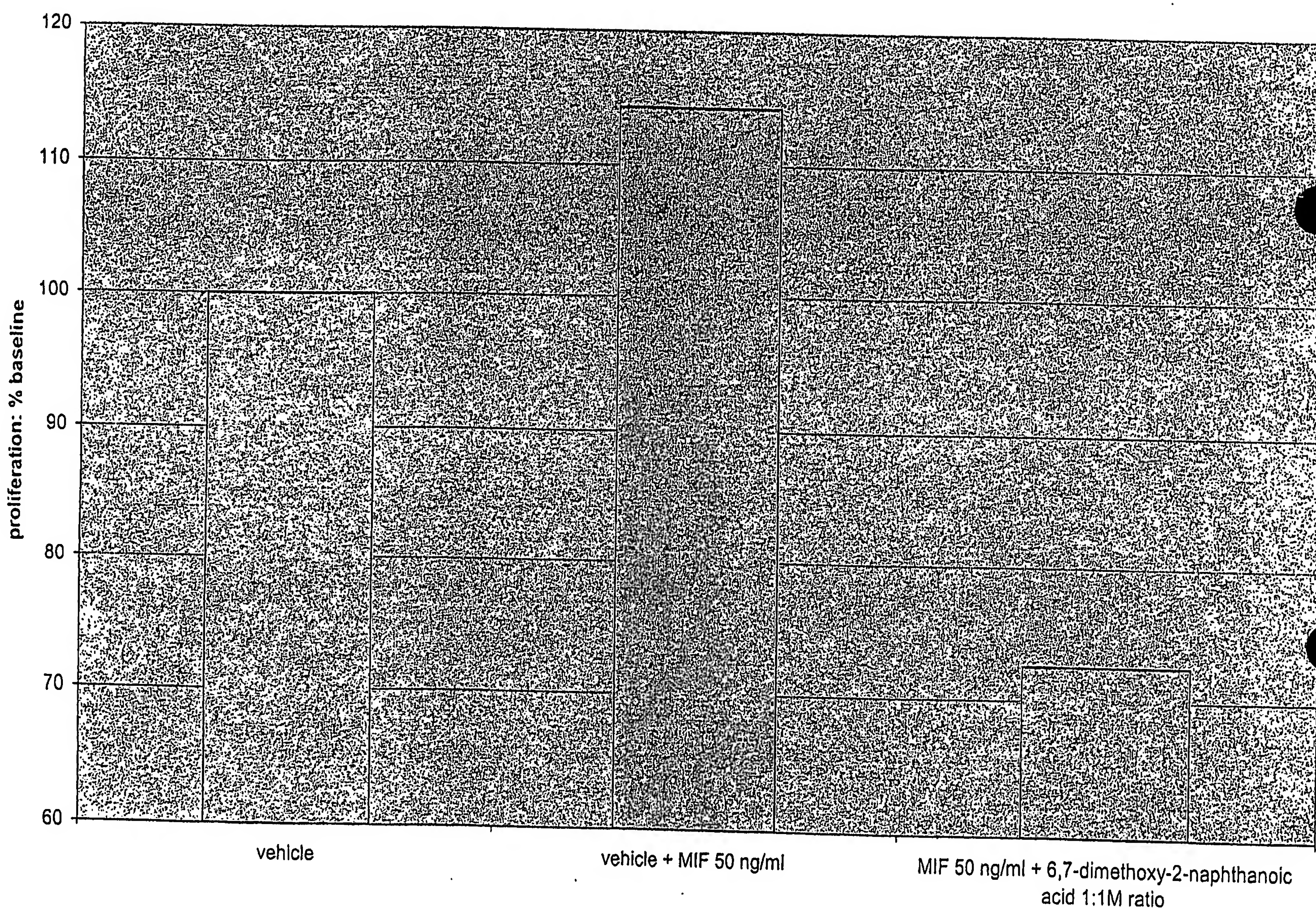

proliferation: % baseline
120
110
100
90
80
70
60
vehicle
vehicle + MIF 50 ng/ml
MIF 50 ng/ml + 6,7-dimethoxy-2-naphthanoic acid 1:1M ratio

Figure 1

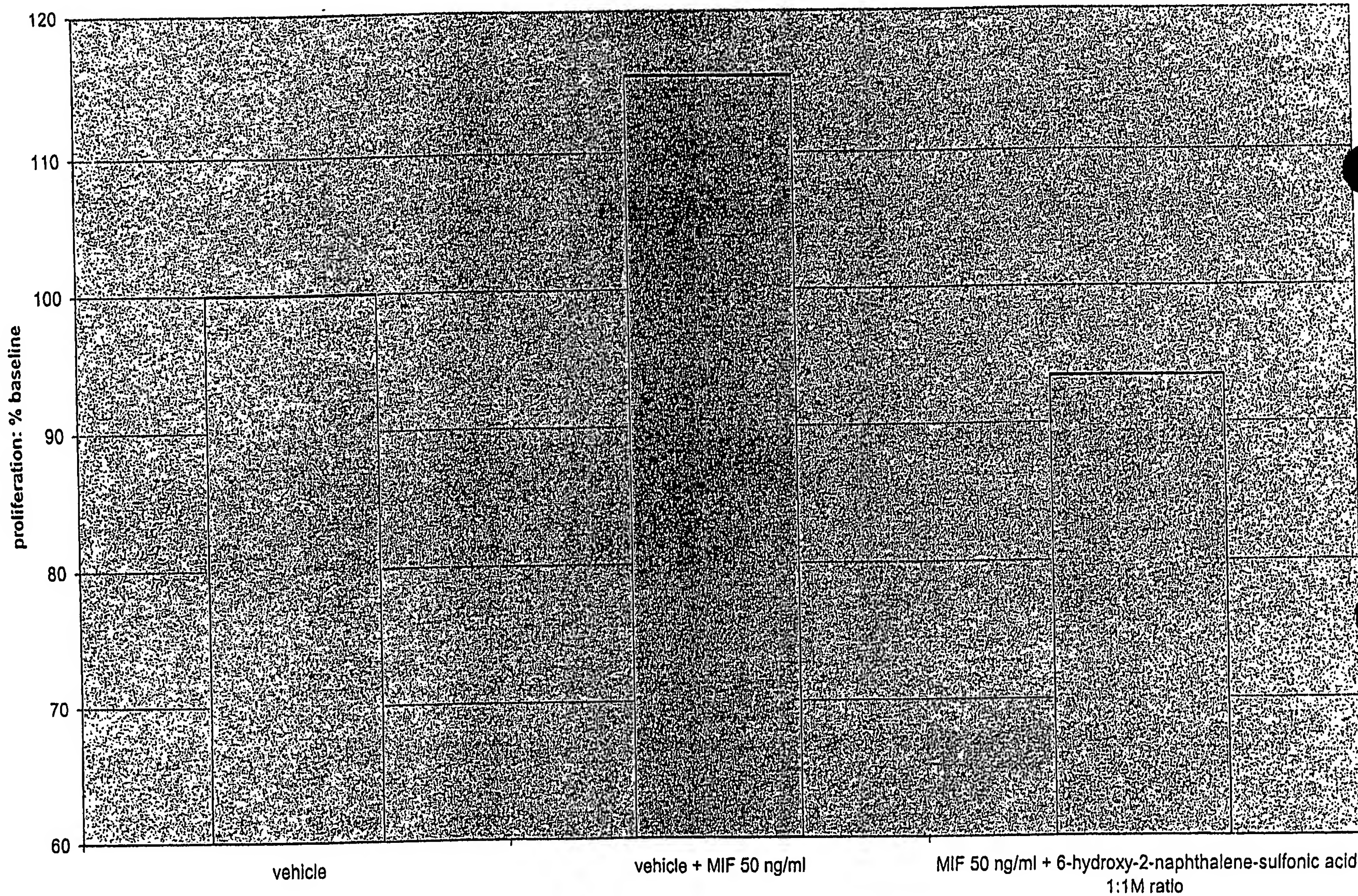
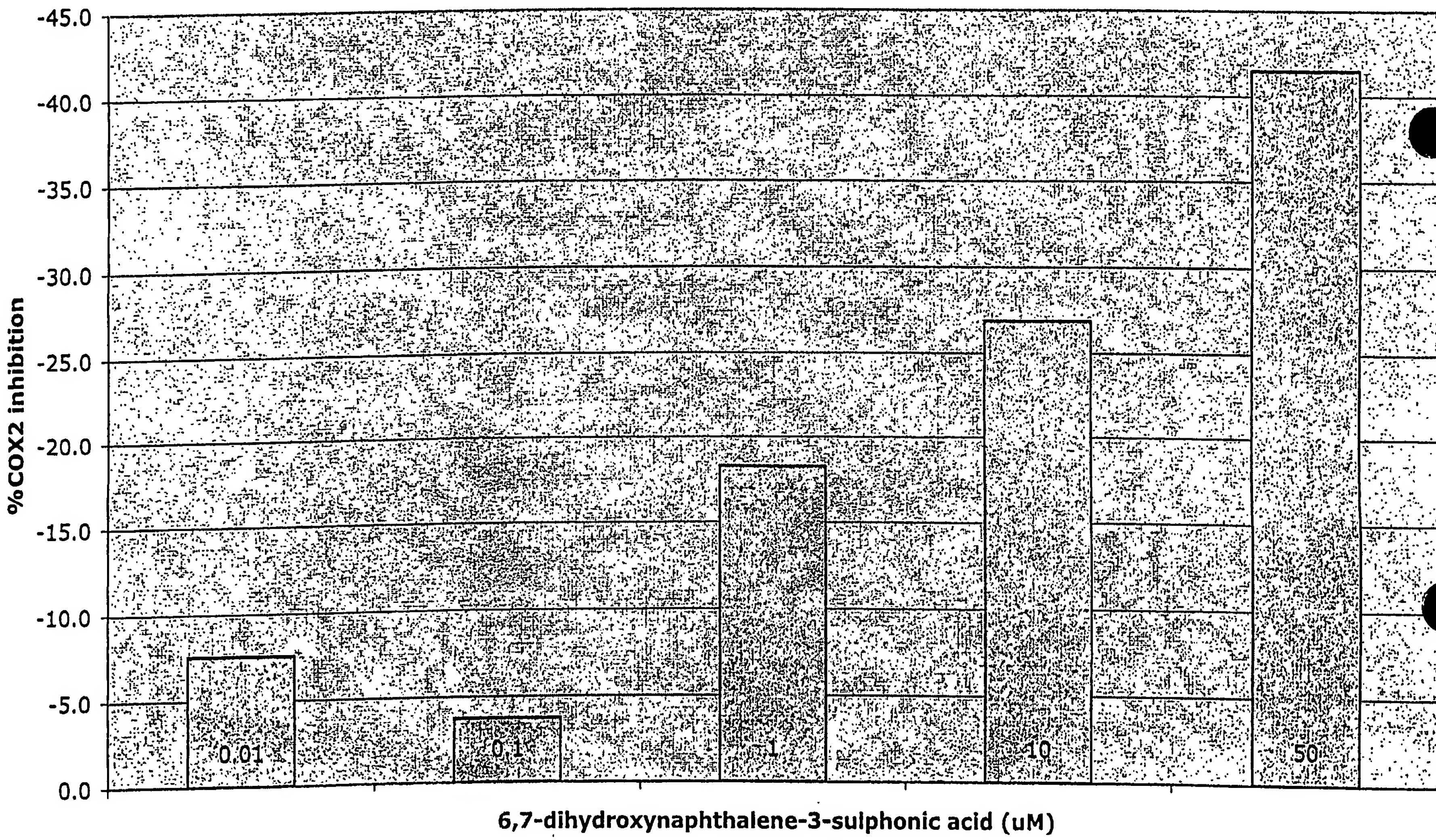

120
110
100
90
80
70
60
proliferation: % baseline
vehicle
vehicle + MIF 50 ng/ml
MIF 50 ng/ml + 6-hydroxy-2-naphthalene-sulfonic acid
1:1M ratio

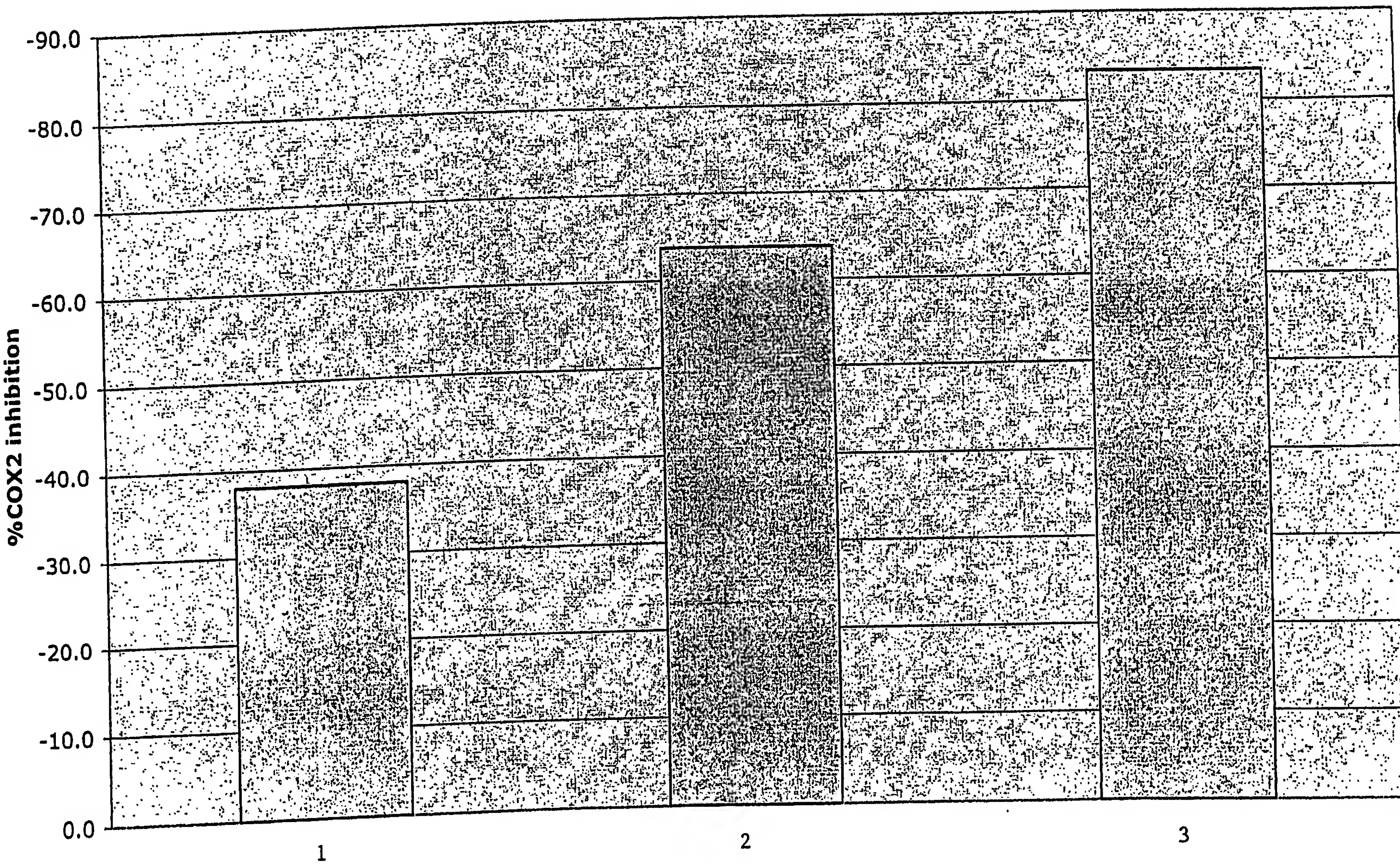
Figure 2

Figure 3



-45.0
-40.0
-35.0
-30.0
-25.0
-20.0
-15.0
-10.0
-5.0
0.0
%COX2 inhibition
0.01
0.1
1
10
50
6,7-dihydroxynaphthalene-3-sulphonic acid (uM)

Figure 4



Rat adjuvant arthritis

Arthritis index: clinical score 0-4 per paw, max=16

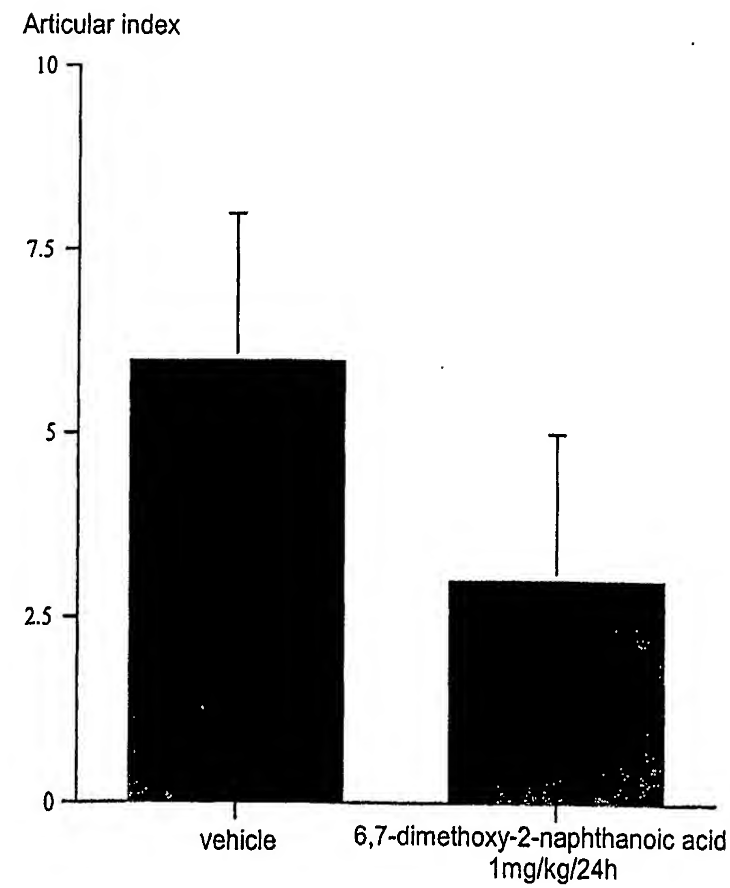


Figure 5

Rat adjuvant arthritis

Synovial fluid (lavage) PMN count

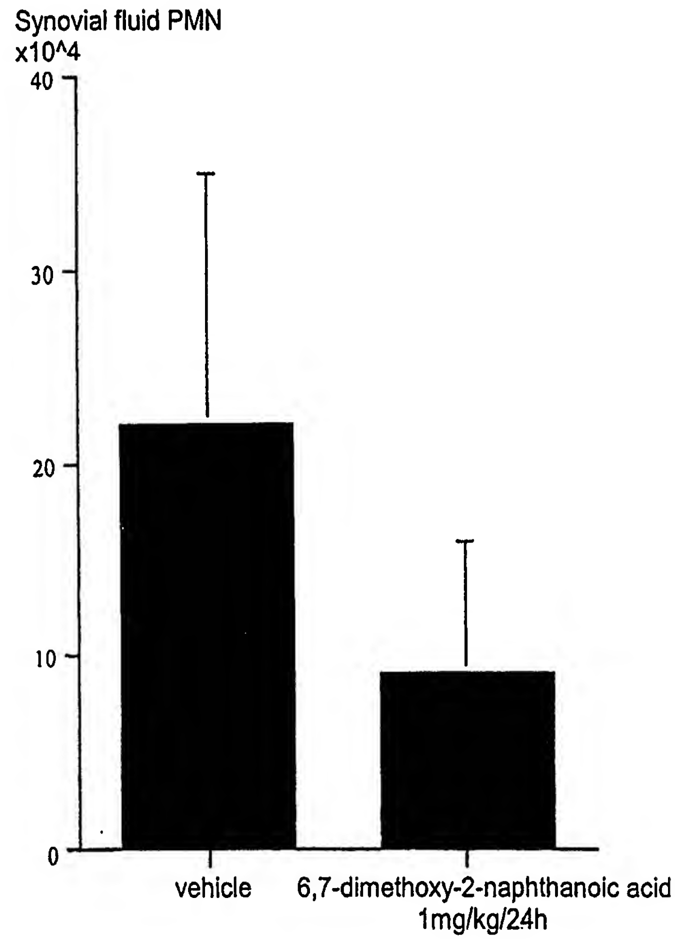
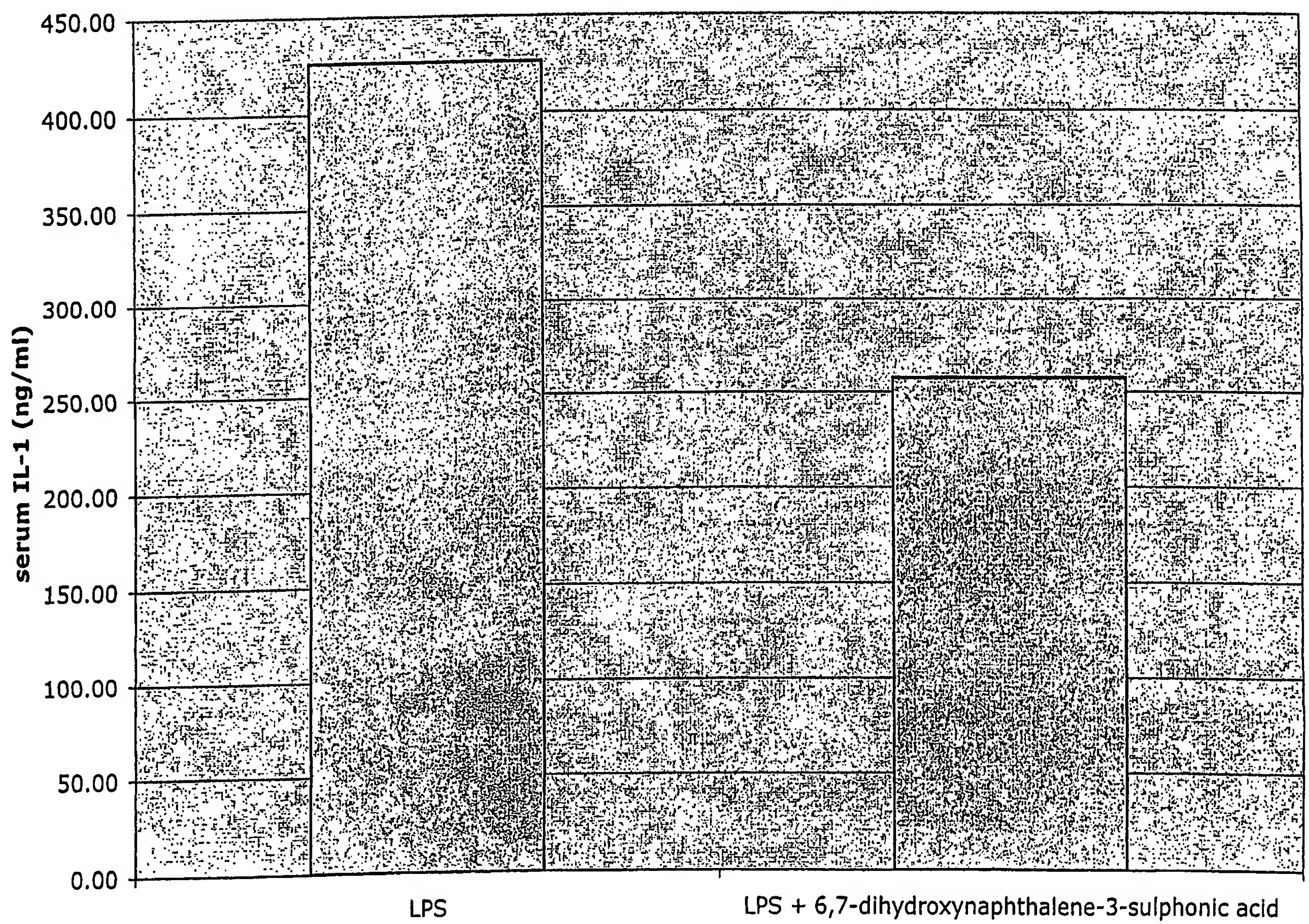


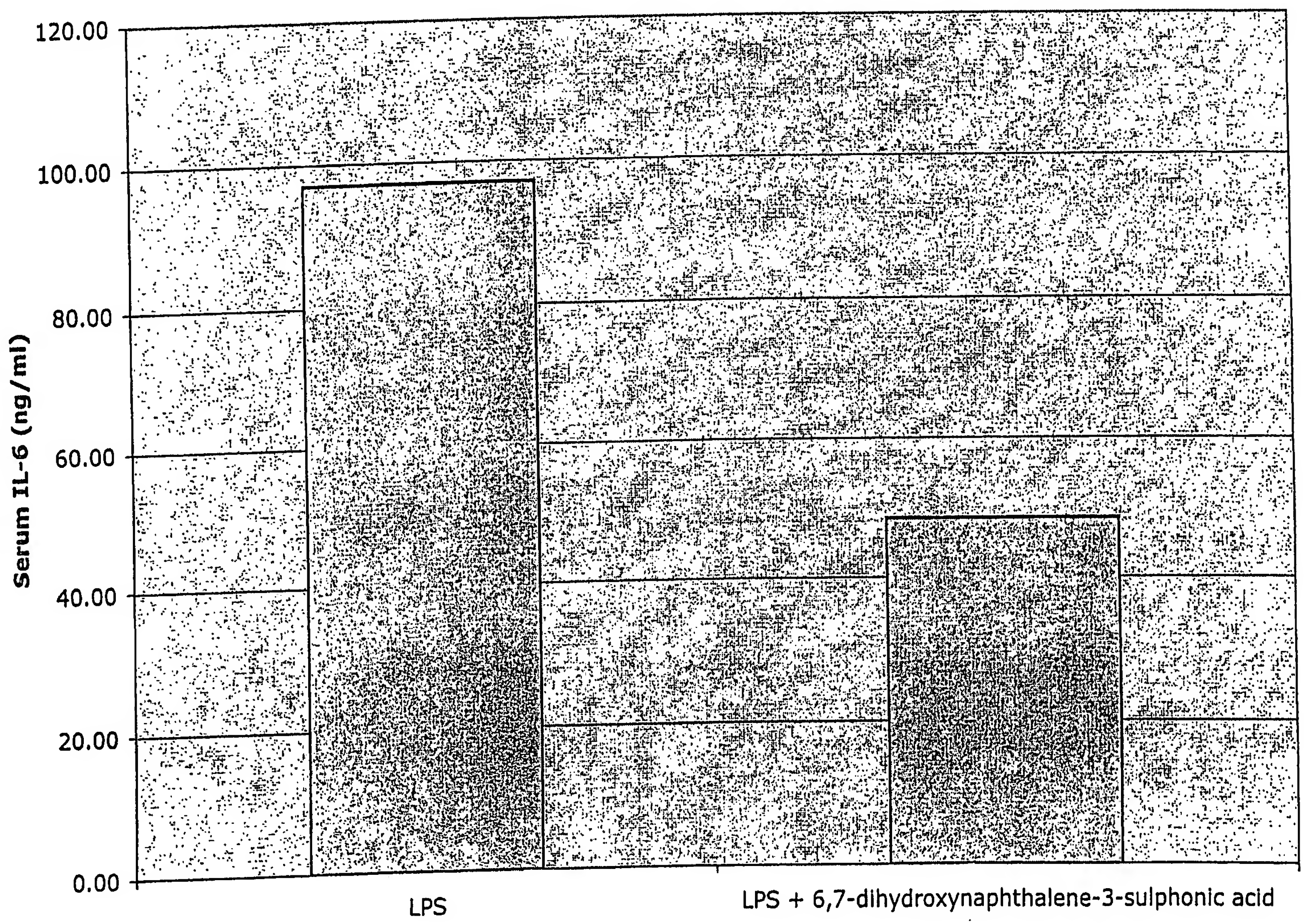
Figure 6

Figure 7



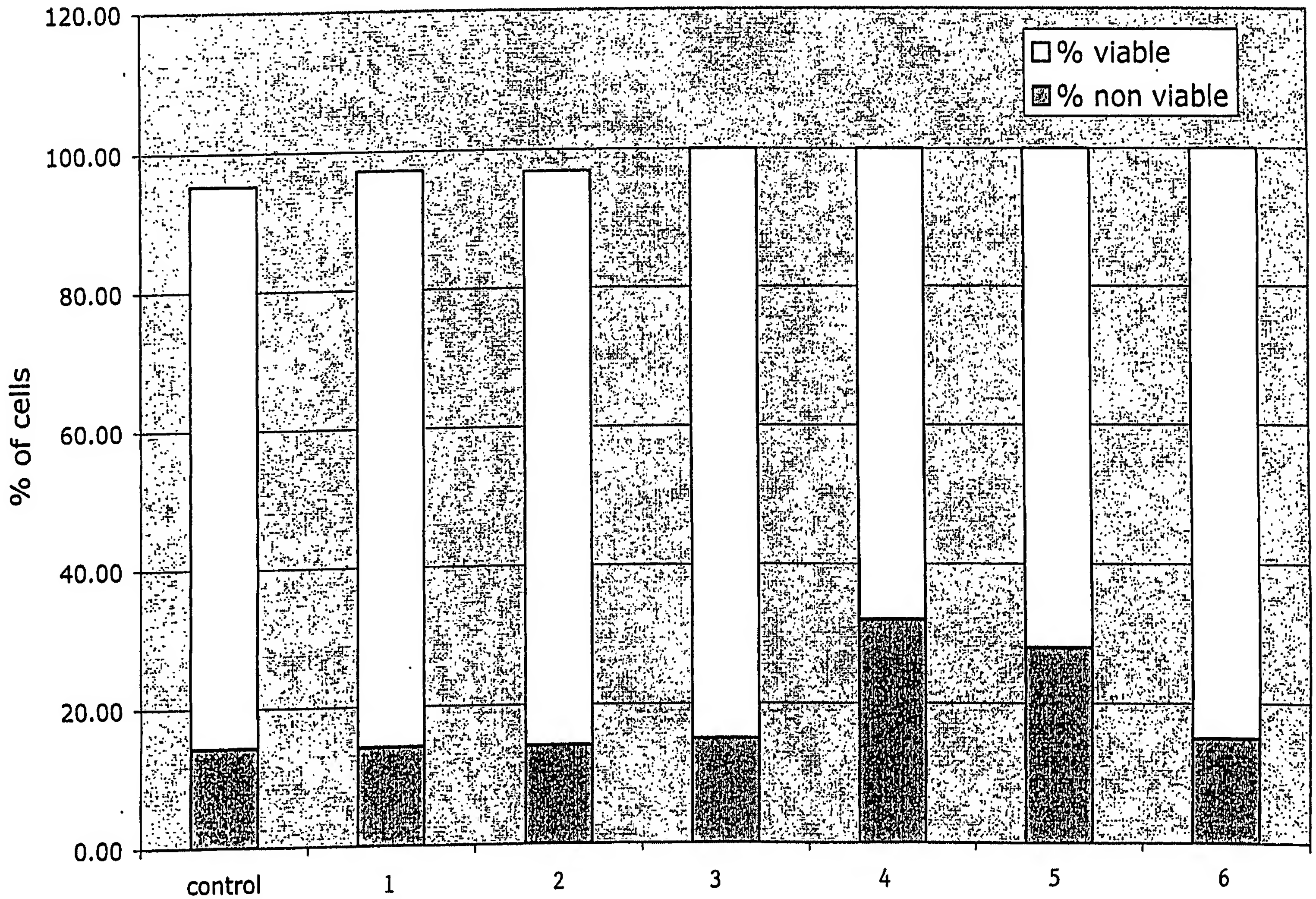
serum IL-1 (ng/ml)
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
LPS
LPS + 6,7-dihydroxynaphthalene-3-sulphonic acid

Figure 8



120.00
100.00
80.00
60.00
40.00
20.00
0.00
Serum IL-6 (ng/ml)
LPS
LPS + 6,7-dihydroxynaphthalene-3-sulphonic acid

Figure 9


% viable
% non viable
% of cells
120.00
100.00
80.00
60.00
40.00
20.00
0.00
control
1
2
3
4
5
6